**Results:** BI 2536 potently inhibited proliferation of NSCLC cell lines with EC50 values of 8 - 14 nM. The compound showed good anti-tumor activity in vivo in all 4 NSCLC xenograft models tested with T/C ("treated/control") values of 1% (Calu-6), 14% (A549), 21% (NCI-H460), and 41% (NCI-H520TC) at doses that were well tolerated. Immunohistochemical analysis of NCI-H460 tumors excised from treated animals as well as near-infrared optical imaging using Cy5.5-coupled annexin V demonstrated mitotic arrest and induction of apoptosis in vivo. Combination therapy with established chemotherapeutic agents (cisplatin, docetaxel or pemetrexed) resulted in improved efficacy in the absence of additional toxicity.

**Conclusion:** BI 2536 is a potent inhibitor of NSCLC cell proliferation in vitro and shows efficacy in multiple human NSCLC xenograft models at well-tolerated doses. Combination therapy with BI 2536 and established chemotherapeutic agents is well tolerated and results in improved efficacy compared to single-agent treatment.

**PD2-1-2** **Cancer Genetics and Tumor Biology, Tue, 16:00 - 17:30**

### Involvement of the tumor suppressor ING2 gene in lung cancer

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**Background:** ING genes (Inhibitor of Growth, ING1-5) are candidate tumor suppressor genes which have been recently identified and characterized. Whereas ING1 expression has been shown to be down-regulated in several tumor types through a yet unknown mechanism (mutation of the gene is a rare event), few data have been reported regarding the status of other members of the family. ING2 is involved in tumor suppression pathways such as apoptosis and senescence. It is a chromatin associated protein, a nuclear phosphoinositide receptor which interacts with trimethylated H3 on lysine 4 and can regulate p53 transcriptional activity by enhancing its acetylation to modulate cellular response to genotoxic stress. ING2 status in human tumors is still unknown (one study reported the reduced expression of ING2 in melanoma) and its role and regulation in carcinogenesis are poorly understood.

**Methods:** We investigated the involvement of ING2 in human lung tumors by analyzing ING2 protein expression on tumors and their related normal tissues (this study was done on samples from the tumor bank (CRB, Grenoble) and with tumor tissue micro-arrays. Immunohistochemical analysis was performed on 75 formalin fixed tumor tissues and normal counterpart using specific ING2 polyclonal antibody KMP1 (kindly provided by Dr. C. Harris) dilution 1/100. Score of immunostaining (0-300) was established by multiplicating the percentage of stained cells (0-100) by intensity (1-3). Cut-off of score for discriminating positive from low expression and negative was determined by the mean level of expression on normal alveolar cells taken as internal control (mean 120): score of 0 to 50 considered as negative; 50 to 120 as low and >120 as positive. mRNA were extracted by Trizol and analyzed by RT-PCR.

**Results:** A strong and mainly (but not exclusively) nuclear staining of ING2 was detected in normal lung tissues, mostly in Type II pneumocytes. In non-small cell lung cancers, a loss or low level of ING2 expression was observed in 75 % (27/36) adenocarcinoma and 82 % (32/39) squamous cell carcinoma as compared with normal lung. Furthermore, in many cases, the ING2 nuclear staining was lost; whereas, the cytoplasmic staining was maintained. These results suggest that in tumor cells ING2 could be excluded from the nucleus. Analysis of mRNA expression by real-time PCR of 13 tumors and corresponding normal lung tissues showed a marked decrease of the mRNA in tumors. Since ING2 promoter contains several CpG islands, methylation of the promoter in these tumors is being investigated.

**Conclusion:** Taken together, these results suggest that ING2 could be inactivated in lung tumors either by downregulation at the mRNA level or exclusion of the protein from the nucleus. Further analysis of these tumors, especially their p53 status should help us to understand the role ING2 could play in lung carcinogenesis.

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**PD2-1-3** **Cancer Genetics and Tumor Biology, Tue, 16:00 - 17:30**

### Changes in plasma levels of cytokines during fractionated radiotherapy of non-small cell lung cancer (NSCLC): the relation with metabolic response and toxicity

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**Background:** Radiation dose escalation might improve local control in NSCLC, but is associated with increased toxicity. Consequently patients have to be selected carefully to prevent, both over- and under-treatment. As part of a prospective clinical trial we reported that metabolic responders and non-responders (EORTC-criteria) showed different time trends in maximal SUV (standardized uptake value, SUVmax) of 18F-FDG on PET-scan. Moreover, metabolic responders showed an improved overall survival compared to non-responders (van Baardwijk et al., Radioth Oncol 2007). The aim of the current study was to investigate changes in plasma levels of cytokines and the association of these changes with SUVmax and the amount of toxicity during and after radiotherapy (RT), to further unravel underlying mechanisms.

**Methods:** From 21 patients included in the clinical trial, repeated plasma samples (n=67) were collected before RT, on day 7 and 14 during RT and 70 days after RT. Levels of IL-1b, IL-5, IL-6, IL-8, IL-10, TNF-α, M30, M65, osteopontin and neopterin were assessed using ELISA's. Unmeasurable levels were assumed to be zero. Levels of cytokines were associated with SUVmax of FDG-PET and toxicity score, according to CTCAE-criteria. Moreover, changes in levels of cytokines between the different time points during RT (day 0 versus day 7, day 7 versus day 14) were correlated with changes in SUVmax within the same time-interval. Results are expressed as mean ± SEM. Pearson correlation coefficient and Mann Whitney U-test were used to analyze the data.

**Results:** A large heterogeneity in evolution in cytokine levels during RT was observed between individual patients. It was shown that changes in SUVmax correlated with changes in the level of IL-6, showing a

correlation coefficient of 0.51 for the change between day 0 and day 7 (p=0.02) and of 0.59 for the change in the second week of RT (day 7 and 14, p=0.02). This correlation was not observed for other cytokines. Moreover, IL-6 levels were higher in patients showing no metabolic response compared to responders: day 0 resp. 18.9 ± 7.6 and 6.0 ± 0.9 (p=0.06), day 7 resp. 31.0 ± 13.0 and 9.5 ± 3.0 (p=0.03) and for day 14 resp. 25.3 ± 12.2 and 8.5 ± 1.9 (p=0.10). Seventy days after RT, levels of IL-1b (r=-0.55, p=0.04) as well as neopterin (r=0.58, p=0.04) were positively correlated with clinically symptomatic pneumonitis (>grade 1). Furthermore, the dyspnea score at this time point showed a graded correlation with plasma levels of neopterin (r=0.76, p<0.01) and the cough score with osteopontin levels (r=0.81, p<0.01), while these phenomena were not observed during RT.

**Conclusions:** Changes in SUVmax were positively correlated with changes in IL-6 levels during radiotherapy, suggesting that changes in FDG uptake might be partly explained by inflammation. Seventy days after radiotherapy, levels of neopterin were correlated with both symptomatic pneumonitis and dyspnea score, assuming cell-mediated immune activation in the development of pneumonitis. Furthermore, levels of osteopontin, a lungfibrosis related protein, were correlated with cough score after radiotherapy.

**PD2-1-4** **Cancer Genetics and Tumor Biology, Tue, 16:00 - 17:30**

### Genome features predict response to chemotherapy for non-small cell lung cancer

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**Background:** While standard chemotherapy for non-small cell lung cancer (NSCLC) confers a survival benefit to patients and improves quality of life, a complete response to therapy is uncommon, especially for patients with advanced disease. Uncovering genomic signatures that predict drug response for specific tumors will allow for the rational selection of effective treatments for individual patients and lay the foundation for personalized treatment strategies.

**Objective:** To determine if genomic features present in pre-treatment tumor cells can predict response to chemotherapy and to identify novel genes associated with drug resistance.

**Methods:** A panel of archived pre-treatment lung tumor biopsies with recorded patient response to a standard doublet regime (cis-platinum/vinorelbine) were selected and subdivided into "responders" and "non-responders" based on clinical parameters. Following pathology review, tumor cells were isolated and DNA was extracted from formalin-fixed, paraffin-embedded tissue sections. Molecular profiling of tumors was undertaken by whole genome tiling-set array comparative genomic hybridization (aCGH). The SeeGH and SIGMA software programs were used for visual analysis of all aCGH profiles. A segmentation algorithm was applied to array data to identify DNA copy number alterations in each tumor. Statistical analysis of processed tumor genome data was used to define regions of significant difference between the "responder" and "non-responder" subgroups.

**Results:** Comparative analysis of high resolution genome profiles for "responder" and "non-responder" tumors revealed segmental genomic gains and losses specific to each group. Genes within these regions of alteration are known to play a role in cellular processes that affect response to chemotherapeutic agents, including DNA repair and apoptotic signalling. Functional experiments for candidate loci are ongoing.

**Conclusions:** High resolution analysis of pre-treatment tumor genomes reveals molecular signatures that can predict response to chemotherapy. Clinical application of such signatures will allow cancer treatments to be tailored to individual patients. In addition, candidate response genes within identified signatures may provide insight into drug resistance mechanisms and serve as novel therapeutic targets.

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**PD2-1-5** **Cancer Genetics and Tumor Biology, Tue, 16:00 - 17:30**

### Specific DNA copy number alterations in arsenic-related lung squamous cell carcinomas

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**Background:** Tobacco smoke is strongly correlated with lung carcinogenesis, particularly for squamous cell carcinoma (SqCC). In addition to tobacco smoke, exposure to arsenical compounds in drinking water has also been strongly related to increased incidence of lung cancer, particularly SqCC.

In the northern part of Chile (Antofagasta City), the population has been chronically exposed to arsenic compounds in drinking water at high concentrations. Antofagasta shows the highest incidence (39.4/100,000 inhabitants) and mortality (33.3/100,000 inhabitants) rates for lung cancer in the country. Almost 70% of diagnosed cases are of the SqCC. However, the prevalence of smoking in Antofagasta is not greater relative to the rest of the country (approximately 20% smokers by population).

The purpose of this study is to compare DNA segment copy number changes in lung SqCC from patients with and without exposure to arsenic and or tobacco smoke to determine pathways that are unique to arsenic induced lung cancer. We also want to determine pathways that are common in the carcinogenesis process irrespective to the type of carcinogen exposure.

**Methods:** A panel of 13 paraffin-embedded lung SqCC was collected from the Hospital of Antofagasta on the basis of patient residence, more than 15 years living in the region prior to diagnosis, and no history of previous cancer. We divided this group in smokers (S-As group) and non-smokers (nonS-As) Twenty-one tumors from non-arsenic exposed patients (S-noAs) were collected from British Columbia Cancer Agency (Canada) for comparison. Genomic profiling was performed by high resolution tiling path array comparative genomic hybridization (CGH). The SeeGH software was used for alignment and analysis of all array CGH profiles. Genomic regions with p-values less than 0.05 (Fisher exact test) were used for analysis.

**Results:** Alterations in NS-As group are much lower than the other two groups. Overall, the frequency of alteration in 3q (the most consistently over-represented region in SqCC related to tobacco smoking) in nonS-As group is lower than in smokers. We also found genomic regions with a high degree of similarity between S-noAs and NS-As e.g. losses at 6q16.1-3, 9q33.1-2 10q11.21 and gains at 12p12.3

NS-As group shows a statically significantly higher frequency of copy number gains of a 4 Mb segment at 19p12. The same region is

 